THE GLOBULIN CONTENT OF THE BLOOD SERUM IN SYPHILIS *

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The combination of refractometry and viscosimetry apparently can be used for the determination of the albumin-globulin ratio in blood serum.

Globulin is in some way associated with the process of immunity, though perhaps only in an incidental way. The Nonne test of the spinal fluid is based entirely on the presence of the globulin. A relation of other tests to globulin is at least suspected. While an increase of globulin in blood serum is not specific for any disease, there may at least be a quantitative relationship to pathologic processes. Hence it would seem that its presence or absence in the body fluids might prove to be of diagnostic significance. Syphilitic blood serum has been chosen for this investigation because it is known to present an increased globulin content. Our study is far from being complete, but the results seem to be sufficiently interesting to warrant a report at this time.

GLOBULIN IN THE BLOOD SERUM IN INFECTIOUS DISEASES

It is a common observation that an infection is followed by an increase of the globulin in the serum. Righetti [1] found an increase on immunizing rabbits with *Bacillus typhosus,* Jacoby [2] after injecting antiricin serum, and Moll [3] after using protein immune serum. Seng,[4]

* After this study was ready for publication, a similar study by Tokuda of Philadelphia appeared in the ARCHIVES OF DERMATOLOGY AND SYPHILOGY (**4**: 512 [Oct.] 1921). The microrefractometric method of Robertson was used in his studies and in general his conclusions coincide with ours.

1. Righetti, H.: An Investigation of the Ratio of Globulins to Albumins in the Blood Serum of Normal Rabbits and of Rabbits Immunized Against Bacillus Typhosus, Berkeley, University of California Press, 1916.

2. Jacoby, M. J.: Ueber Ricin-Immunität, Braunschweig, F. Vieweg und Sohn, 1901.

3. Moll, L.: Ueber künstliche Umwandlung von Albumin in Globulin, Beitr. z. chem. Phys. u. Path. **4**:563-577, 1903.

4. Seng, W.: Ueber die qualitativen und quantitiven Verhältnisse der Eiweisskörper im Diphtherieheilserum, Ztschr. f. Hyg. u. Infectionskrankh. **31**:513, 1899.

Atkinson,[5] Joachim,[6] Meyer, Hurwitz and Taussig[7] studied the globulin in animals immunized against diphtheria, and Hurwitz and Taussig in animals immunized against tetanus and botulism. Langstein and Mayer[8] and Hurwitz and Meyer[9] found an increase after injecting living, or killed, bacteria. An increase of leukocytes seemed to accompany an increase in globulin, and this fact was believed to furnish new evidence for the theory that the globulin is derived from a destruction of the white cells. The study of Hurwitz and Meyer is particularly interesting because it tends to corroborate this observation. They found that the rise of globulin may apparently antedate any increase in resistance, and that animals possessing "basic" immunity show a more rapid rise than those in which this immunity is less marked. The latter fact has been corroborated recently by the work of McDonagh,[10] who says:

> The formation of complement is the one protective mechanism against all invading micro-organisms. It is a lipoid globulin and an oxidizing ferment. Microorganisms are destroyed by hydrolysis and oxidation of their lipoid globulin through fermentation. In order that the complement may be able to bring its ferment action to bear on the particular micro-organism, it must adapt itself to it by rearranging and building up its lipoid globulin until it has a stereochemical and molecular configuration homologous with that of the parasite. This is accomplished by absorption of the lipoid globulins that have been discharged into the plasma by the lymphocytes. The initiation of the formation and discharge of this substance in the case of syphilis is due to the activity of the spirocheta, but its continuation is not necessarily dependent upon the presence of living parasites. If the infection is severe the "reagin" production is continuous throughout the whole life. Arsphenamin increases the amino content of syphilitic sera due to breaking up of lipoid globulins.

The process of immunity seems to demand an increase of globulin through a probable liberation from the leukocytes. The initiation of this process is due to a bacterial toxin, but the process progresses

5. Atkinson, J. P.: The Fractional Precipitation of the Globulin and Albumin of Normal Horses' Serum and Diphtheria Antitoxic Serum, and the Antitoxic Strength of the Precipitates, J. Exper. M. **5**:67-75, 1900-1901.

6. Joachim, J.: Ueber die Eiweissvertheilung in menschlichen und thierischen Körperflüssigkeiten. Arch. f. Physiol. **93**:558-604, 1903.

7. Meyer, K. F.; Hurwitz, S. H., and Taussig, L.: Studies on the Blood Proteins. III. Albumin-Globulin Ratio in Antitoxic Immunity, J. Infect. Dis. **22**:1-27, 1918.

8. Langstein, L., and Mayer, M.: Ueber das Verhalten der Eiweisskörper des Blutplasmas bei experimentellen Infektionen, Beitr. z. chem. Physiol. u. Path. **5**:69-82, 1903.

9. Hurwitz, S. H., and Meyer, K. F.: Studies on the Blood Proteins. I. The Serum Globulins in Bacterial Infection and Immunity, J. Exper. M. **24**:515-546, 1916.

10. McDonagh, J. E. R.: The Biology and Treatment of Venereal Diseases, London, Harrison and Sons, 1915.

according to the response of the organism as a whole. The more rapid rise of globulin in animals possessing "basic" immunity may be due to an easier liberation of the globulin. The action of arsphenamin on the globulin is very definite, according to McDonagh, and it may well be explained by the more rapid breaking down of the globulin compound into amino and fatty acids.

Atkinson [5] states that all the antitoxic power is carried by the globulin. Some findings seem to prove, however, that the globulin rise need not necessarily follow or precede immunization. Glässner [11] was able to keep the ratio unchanged by the injection of small amounts of toxins into the animal over a long period of time. He believes that an increase is observed only when very marked metabolic disturbances occur. His series is small and he had made only one determination before the immunization, and one after. Twelve years later, however, a similar observation was published by Meyer, Hurwitz and Taussig,[7] who state that "A rise in globulin may be considered to be a manifestation of an upset in the delicate protein balance of the blood." In some of their experiments, however, they could not find a relation between the increase of globulin and the antitoxic potency of the serum.

Interesting observations on the globulin content in syphilitic serums have been made by Noguchi,[12] who found that in 107 cases of active syphilis, primary, secondary and tertiary, the globulin content of the serum was commonly higher than normal. He could not establish any relation between the amount of globulin and the strength of the Wassermann reaction. He observed a reduction of the globulin after administration of mercury and arsphenamin. The points drawn from the investigations cited have a definite relation to our subject.

1. An increase in the serum globulin is practically always associated with the process of immunization.

2. The amount of globulin is not always proportional to the immunity. In fact, careful immunization of an animal occasionally may not be followed by an increase of globulin.

3. The increased production of globulin usually starts with the infection, but it may proceed even after the infection has been overcome.

4. Globulin is more readily formed in cases in which a basic immunity exists. The rate of formation of globulin may, under certain conditions, be an indication of the individual's reaction and the ability to bring about self-immunization.

11. Glässner, K.: Ueber das Verhalten des Blutglobulins beim Immunisirungsvorgange, Ztschr. f. exper. Path. u. Therap. **2**:154-160, 1905-1906.

12. Noguchi, H.: Die quantitative Seite der Serodiagnostik der Syphilis, mit Bermerkungen über den Globulin- und natürlichen Antihammel-Ambozeptorgehalt syphilitischer Sera, sowie über die angebliche Gefahr von Auftreten des Neisser-Sachschen Phänomens beim Verwenden des antimenschlichen Ambozeptors, Ztschr. f. Immunitätsforsch. u. exper. Therap., Orig. **9**:715, 1911.

5. In syphilis, the rise in globulin is an especially marked feature.
6. Arsphenamin has a definite influence on the globulin content.

METHODS OF STUDY

Globulin is obtained from blood, after its removal from the body, by fractioning the protein in the serum into two definite substances, albumin and globulin. Further fractions have been observed, but these two substances are well enough defined to warrant study directed to them alone.

The absolute amount of each substance might be studied, although this necessarily fluctuates with the total amount of protein, which is subject to rather great variations. The study of the relationship of albumin and globulin seemed to be of more value because variations in it are probably more indicative of changes of biologic significance.

The older methods of separating albumins and globulins are all based on chemical division and separate quantitative determinations of the different constituents of the serum. Ammonium sulphate is used for the precipitation of the globulins, dialysis for the removal of the nonproteins, and heat for the coagulation of the protein. Each process requires a well trained chemist because stress is laid on the quantitative side.

METHODS USED IN DETERMINING THE PROTEIN CONTENT OF THE BLOOD

1. The gravimetric method, in which the amount of precipitated and dried protein is determined, is a very exacting and time-consuming procedure. It was used by most of the older investigators.

2. Determining the nitrogen after Kjeldahl and multiplying the result with 6.25 (an arbitrary factor) naturally gave only approximate results.

3. The refractivity method used by Reiss [13] gives good indirect information of the total protein content.

4. Robertson [14] also uses Reiss' method. He isolates the various protein fractions and determines separately the refractive index of each.

5. Autenrieth [15] has applied the colorimetric method. After separation, the proteins are redissolved and a yellow color is obtained by

13. Reiss, E.: Die refraktometrische Blutuntersuchung und ihre Ergebnisse für die Physiologie und Pathologie des Menschen, Ergebn. d. inn. Med. u. Kinderh. **10**:531-634, 1913.

14. Robertson, T. B.: On the Refractive Indices of Solutions of Certain Proteins. VI. The proteins of Ox-Serum; a New Optical Method of Determining the Concentrations of the Various Proteins Contained in Blood-Sera, J. Immunol. **11**:179, 1912. A Micro-Refractometric Method of Determining the Percentages of Globulin and Albumin in Very Small Quantities of Blood Serum, J. Immunol. **22**:233-239, 1915.

15. Autenrieth, W.: Ueber kolorimetrische Bestimmungsmethoden: Die Bestimmung von Serumalbumin und Globulin im Harn, in der Aszitesflüssigkeit und im Blutserum, München. med. Wchnschr. **64**:241, 1917.

adding sodium hydroxid and copper sulphate. This color is, in its intensity, fairly proportional to the concentration of protein, and may be compared with a standard color.

These methods are rather time-consuming and some of them are none too accurate.

Previous Results.—The albumin-globulin ratios determined by other investigators are given in Tables 1, 2 and 3. Compared with the importance of the matter, the small number of investigations is remarkable. This is due to the fact that all the methods used previously were time-consuming and difficult. Besides, most of the figures found

TABLE 1.—ALBUMIN-GLOBULIN RATIO IN NORMAL ANIMALS

Observer and Reference	Animal	Albumin	Globulin
Thompson: J. Biol. Chem. **20**: 1, 1915	Turkey	84	16
	Rooster	81	19
Wells: J. Biol. Chem. **15**: 37-41, 1913	Rabbit	79	21
Righetti: Footnote 1	Rabbit	78	22
Thompson	Hen	77	23
	Duck	74	26
	Goose	74	26
Briggs: J. Biol. Chem. **20**: 7-11, 1915	Pigeon	72	28
	Guinea fowl	70	30
	Rooster	62	38
Hammarsten: Arch. f. Physiol. **17**: 413-468, 1878; **18**: 38-116, 1878; Ztschr. f. Physiol. Chem. **8**: 467-502, 1883-1884	Ox	58	42
Joachim: Footnote 6	Hen	56	44
Robertson: Footnote 15	Ox	55	45
Joachim: Footnote 6	Horse	50	50
	Cow	46	54

TABLE 2.—ALBUMIN-GLOBULIN RATIO IN NORMAL HUMAN BEINGS

Observer and Reference	Blood Specimen	Albumin			Globulin		
		Highest	Lowest	Average	Highest	Lowest	Average
Joachim: Footnote 6	Man	70	53	61.0	47	30	39.0
Hammersten: Arch. f. Physiol. **17**: 413-468, 1878; **18**: 38-116, 1878; Ztschr. f. Physiol. Chem. **8**: 467-502, 1883-1884	Man	..	..	61.5	..	..	38.5
Epstein: J. Exper. M. **16**: 719-731, 1912	Man	..	..	63.0	..	..	37.0
Lewinski: Arch. f. d. ges. Physiol. **100**: 611-633, 1903	Man	68	58	63.0	42	37	37.0
Joachim: Footnote 6	Fetus	..	..	66.0	..	..	34.0
Alder: Footnote 19	Man	80	55	68.0	45	20	32.0
Hoffman: Arch. f. exper. Path. u. Therap. **16**: 133-142, 1883	Man	72	55	68.0	35	28	32.0
Joachim: Footnote 6	Placenta	..	..	70.0	..	..	30.0
Tranter and Rowe: J. A. M. A. **65**: 1433-1434 (Oct. 23) 1915	Man	89	67	78.0	33	11	22.0

in the literature are based only on a small number of determinations and have, therefore, only a relative value.

In Table 1 the wide range of the ratio in different animals is demonstrated. The albumins are almost always preponderant, especially in smaller animals. In the blood of the ox, the horse, and the cow, the albumins were found to constitute about one half of the serum protein.

Table 2 demonstrates the fact that in normal human serum the albumin-globulin ratio may be subject to marked variations, although

the average percentage of globulin ranges only between 22 and 39 per cent. It may be as low as 11 per cent. The highest is 47 per cent.

Table 3 is incomplete, but it may be seen that even in disease the globulin percentage is seldom higher than 50; a lower percentage may be considered within normal limits.

TABLE 3.—ALBUMIN-GLOBULIN RATIO IN CERTAIN DISEASES

		Albumin	Globulin
Hurwitz and Lucas*.....	Hemophilia.....	73	27
Epstein.....	Interstitial nephritis.....	64	36
Epstein.....	Mixed form of nephritis.....	57	43
Epstein.....	Pneumonia.....	56	44
Epstein.....	Diabetes mellitus.....	51	49
Epstein.....	Emphysema.....	50	50
Epstein.....	Cardiac conditions.....	48	52
Epstein.....	Paranchymatous nephritis.....	11	89
Noguchi.....	Tuberculosis.....		Increased
Noguchi.....	Hodgkin's disease.....		Increased
Noguchi.....	Cancer.....		Increased

* Hurwitz, S. H., and Lucas, W. P.: A Study of the Blood in Hemophilia, Arch Int. Med. **17**: 543-569, 1916.

METHOD APPLIED IN THE STUDY

Naegeli's [16] method was used. It is very different from those described previously, and introduces an entirely new technic. Its applications and limitations have been fairly well worked out by the assistance of Naegeli, Heyder,[17] Rohrer [18] and Alder.[19]

Determination of the Albumin Ratio with the Aid of Viscosimetry and Refractometry.—Two important facts form the basis of Naegeli's method for the determination of the albumin-globulin ratio: First, the viscosity and refractivity of the serum depend almost entirely on the protein content. Second, with some exceptions the other constituents, such as salts and sugar, have a practically negligible influence, owing partly to their relatively small amount and partly to the remarkable constancy.[20] Viscosimetry and refractometry combined

16. Naegeli, O.: Blutkrankheiten und Blutdiagnostik, Berlin, 1919.

17. Heyder: Bestimmung der Refraktion und Viscosität von Globulin und Albuminen in ihren Mischungen nach Verscheidenen Verhältnissen, Inaug. Diss., Tübingen, 1915.

18. Rohrer, F.: Bestimmung des Mischverhältnisses von Albumin und Globulin in Blutserum, Deutsch. Arch. f. klin. Med. **121**:221-240, 1916; Physiol. Abstr. **2**:116, 1917.

19. Alder, A.: Die physiologischen Schwankungen des Mischungsverhältnisses von albumin und Globulin im menschlichen Blutserum, Deutsch. Arch. f. klin. Med. **126**:61-72, 1918.

20. In the refractometric determination of the total proteins, the nonprotein substances are represented by a constant factor of 0.00277, which is subtracted from the refractometric index of the screen.

have been used in the determination of the total protein content of the blood and the results have compared favorably with other methods. This is not true for the viscosimetric determinations alone in which a marked discrepancy could be demonstrated between different serums which were known to contain like amounts of total protein (Alder,[19] Bircher [21]).

It was found that solutions of proteins which were relatively more viscous than other solutions, but with the same refractivity, contained a higher proportion of globulins. Definite mixtures of albumin and globulin had a viscosity always higher than that of an albumin solution of the same refractivity and lower than the viscosity of a corresponding

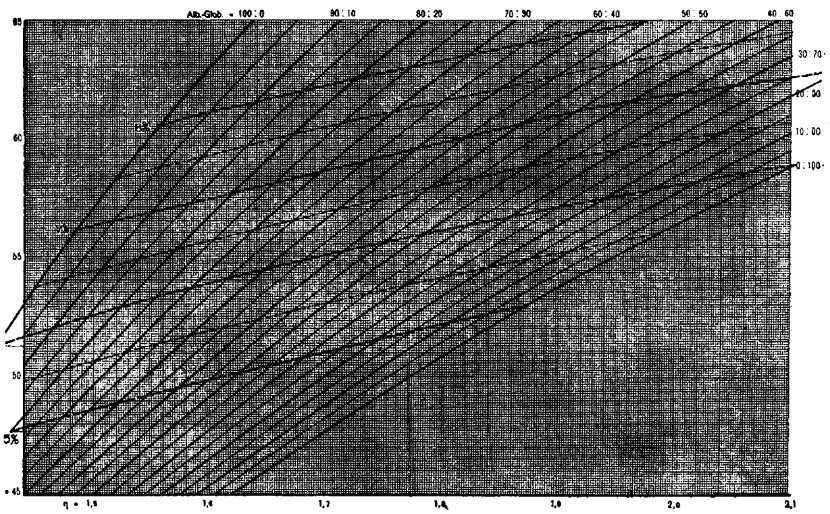

Fig. 1.—Viscosimetric and refractometric function of albumin-globulin mixtures (chart used at the clinic of the University of Zürich). This chart allows the interpretation of an unknown mixture of albumin and globulin when the viscosity and the refraction of the serum have been determined. The dotted lines represent the viscosimetric-refractometric function of albumin-globulin mixtures of equal concentrations.

globulin solution. In Figure 1 one of Naegeli's charts, which has been constructed with the aid of these findings, is reproduced. A discussion of this chart is found in Naegeli's book and in a paper on viscosity by Bircher.[22]

21. Bircher, M. E.: Die Beziehung zwischen der Viscosität des Blues und dessen Gehalt an Blutkörperchen und gelöstem Eiweiss, Arch. f. Physiol. **82**:1-27, 1920.

22. Bircher, M. E.: Clinical Diagnosis by the Aid of Viscosimetry of the Blood and the Serum, with Special Reference to the Viscosimeter of W. R. Hess, J. Lab. & Clin. M. (to be published).

Reiss and Robertson have made some interesting observations on the protein content of the blood as determined by the refractometer.

Reiss found a refractive value corresponding to 1 per cent. protein of 0.00172. After substracting from the refractivity of the total serum the refractivity of distilled water, which is 1.33320 at 17.5 C., and the refractivity of the nonproteins, which is 0.00277, he divides the remainder by 0.00172 in order to obtain the percentage of total protein contained in the serum. He has checked the method with numerous others and found it very satisfactory. He states that the ratio of the albumins and globulins is practically constant and, therefore, does not affect the results. Accepting this statement, one may conclude from Naegeli's chart that the lines of identical refractivity represent also identical percentages of protein. Knowing the albumin-globulin ratio, it should be easy to determine the amount of albumin or globulin in the serum. After studying the work of Robertson we refrained from using the percentages. Robertson accurately determined the refractometric value of different proteins which he found was, for the globulins 0.00227 and for the albumin 0.00183. The difference is marked and must have a noticeable effect on the refractivity of the combined protein. It is hard to understand why both values are higher than the one of Reiss, and Robertson claimed for a long time that for this reason the refractivity of the nonprotein must be lower than 0.00277. Only recently he confirmed the finding of Reiss. The question will remain until further studies can give a satisfactory answer. Reiss published figures for the albumins and globulins which are almost identical with those of Robertson. If these figures are used in the determination of the percentage of total protein, the albumin-globulin ratio must be taken into consideration. The refractive index for a given percentage of total protein can be calculated; and for each ratio a different value is found, the lowest for a pure albumin solution, the highest for a globulin solution. These calculations have been carried out by us and are reproduced in Naegeli's chart by dotted lines. They need experimental confirmation, because the values of Reiss and Robertson are not absolutely accurate. This addition to Naegeli's chart is necessary for the determination of the total protein as well as of its component fractions.

It has been demonstrated that the viscosity as well as the refractivity depends on the albumin-globulin ratio. The refractivity, however, is less influenced by a change in this ratio than the viscosity. This discrepancy is the basis of Naegeli's chart and can be expressed graphically by diverging curves. The field covered by these curves represents this difference.

Determination of the Viscosity.—With the aid of the viscosimeter of Hess,[23] the principles and uses of which I have described, a com-

23. Hess, W. R.: Quoted by Bircher (Footnote 22).

parison is made between the flow-through-volume of water and that of the serum. The dimensions of the tube, the pressure, the time, and the temperature are the same, and according to the law of Poiseuille the viscosities of two fluids are inversely proportional to their flow-through-volume. The instrument of Hess is so simple in its construction that the relative viscosity may be read immediately. Only one drop of serum is needed, and the determination can be carried out in less than one minute.

TABLE 4.—COMPARISON OF PULFRICH UNITS WITH REFRACTIVE INDEX AT 17.5 C.

Reading of Refractometer*	Pulfrich Units	Index of Refraction	Reading of Refractometer*	Pulfrich Units	Index of Refraction
65° 10	48.4	1.34590	64° 30	57.3	1.34920
9	48.6	595	29	57.6	928
8	48.8	603	28	57.8	936
7	49.1	611	27	58.0	944
6	49.3	620	26	58.2	952
5	49.5	628	25	58.5	960
4	49.7	636	24	58.7	968
3	49.9	644	23	58.9	976
2	50.2	652	22	59.1	984
1	50.4	661	21	59.3	992
65° 0	50.6	1.34669	64° 20	59.5	1.35000
59	50.8	677	19	59.7	008
58	51.1	685	18	60.0	017
57	51.3	694	17	60.2	025
56	51.5	702	16	60.4	034
55	51.8	710	15	60.6	043
54	52.0	718	14	60.8	051
53	52.2	727	13	61.1	059
52	52.4	736	12	61.3	068
51	52.7	744	11	61.6	076
64° 50	52.9	1.34752	64° 10	61.8	1.35085
49	53.1	763	9	62.0	093
48	53.4	771	8	62.2	101
47	53.6	780	7	62.5	109
46	53.8	788	6	62.7	117
45	54.1	796	5	62.9	125
44	54.3	804	4	63.1	134
43	54.5	812	3	63.3	142
42	54.7	821	2	63.5	150
41	54.9	829	1	63 8	158
64° 40	55.1	1.34837	64° 0	64.0	1.35166
39	55.3	847	59	64.2	174
38	55.6	854	58	64.5	183
37	55.8	862	57	64.7	191
36	56.0	870	56	64.9	200
35	56.2	878	55	65.1	208
34	56.5	887	54	65.4	216
33	56.7	896	53	65.6	225
32	56.9	903	52	65.8	233
31	57.1	912	51	66.1	242
			63° 50	66.3	250

* Applies only to the instrument used in this investigation.

Determination of the Refractivity.—Any instrument which permits the determination of the refractive index may be used for this method, although the immersion refractometer of Pulfrich as described by Reiss and Naegeli is the simplest. It is graduated on an arbitrary scale into so-called Pulfrich's units, which have been used in the charts. Table 4 compares these units with the refractive index at 17.5 C., so that the units may easily be obtained from the refractometer reading.

The usual refractometer requires three drops of serum. The reading is made immediately and after the receptacle is carefully cleaned the next determination may be made.

Discussion of the Method.—The method is very delicate and would entirely lose its value if the viscosimetry and refractometry were not accurate. With a little practice, the second decimal of the viscosity may be determined, which is absolutely necessary. The smallest refractometric unit which it is necessary to read is one minute, and this lies within the limit of experimental error. Two facts are of the greatest importance as evidence of the reliability of the tests:

1. The great constancy of the ratio in the same individual over a certain length of time, as shown by Naegeli. This proves that the technical error can be only very small, and that it must depend on certain well defined substances.

2. The numerous observations of Naegeli on normal blood gave a ratio which favorably compared with values for albumin and globulin found by other methods and other investigators. This is the chief evidence that the values found represent the amount of albumins and globulins in man.

> Whether the precipitate (with magnesium sulphate) comprises all of the globulin or not, the constancy of the value in solution shows that it is either pure globulin or a mixture of perfectly definite and invariable composition, provided the conditions of precipitation are strictly adhered to. If the proportion of this substance is different in the serum of different individuals, we may be fairly confident, therefore, that the quantitative relations of the globulin and albumin groups are different in these animals (Robertson).

Naegeli's method is not an absolutely accurate test, but Naegeli has demonstrated in his large number of examinations that a clinical application is justified. We have always been astonished by the great constancy of the findings in experiments on the same individual, extending over a period of several weeks. An indirect method can never claim to be the most accurate, but the direct analytic methods in this special field have proved unsatisfactory, owing to the difficulty in precipitating quantitatively the globulin, and to the great amount of delicate work involved and the time consumed.

The advantages of the method used by us are: (1) the short time consumed by the determination (five minutes); (2) the simplicity of the manipulations, which can be carried out by any one after short practice; (3) the small amount of serum required, which is even smaller than that required by the method of Robertson, and (4) the fact that the serum does not undergo chemical change. The questions as to whether or not all the globulins have been precipitated and whether some albumins have been precipitated at the same time do not enter into consideration. On the contrary, it is quite certain that the entire substances in question exert their specific physical influence in refracting the light or in increasing the internal friction.

TECHNIC

Selection.—The patients from whom blood samples were taken for the present study were all under treatment for syphilis in the section on dermatology and syphilology in the Mayo Clinic. The stage of the disease and its manifestations and activity, as far as they can be recognized by clinical study, and by the aid of the Wassermann reaction and of the cerebrospinal fluid test, were carefully noted.

The conditions under which the blood was obtained were the same throughout the entire study. The patient entered the hospital the evening before the examination. He received a small breakfast and was advised to rest during the morning, either in bed or in a chair. The noonday meal consisted of a glass of milk and a cracker. About three hours later the blood was drawn. Food intake and physical exercise, the two most important factors which influence the protein content of the serum, could, therefore, have no influence on the test. Although Naegeli and others found that the albumin-globulin ratio is much more constant than the protein content and that it does not depend on the foregoing factors, they were none the less taken into consideration.

Method of Bleeding.—A few fundamental conditions must be observed. The sample must be obtained from blood which is in actual circulation. Sluggish capillary blood or blood obtained after prolonged venous compression has lost a part of its water content and, under certain conditions, of its albumin content also. The blood must be protected from any contamination, especially with water, when a syringe is used, since hemolysis will be produced. Evaporation is avoided by carefully closing the tubes containing the blood samples. (Other methods of obtaining blood are described by Reiss and Naegeli.) The blood in our series was withdrawn just before the intravenous administration of arsphenamin. At the point of introduction of the large therapeutic needle, a small infiltration of procain is made and, after compression of the veins, the needle is introduced. Since the needle has a caliber not much smaller than that of the vein, an instantaneous outflow of blood is secured, and thus the vein is relieved from the compression above. After the outflow of several cubic centimeters of blood, which may have suffered a change due to the compression, a sterilized Wassermann tube is placed at the opening of the needle and from 2 to 5 c.c. of blood collected. The examination can be carried out with 1 c.c. but the larger amount gives the opportunity to check the findings. The withdrawal of the blood requires only a few seconds. In this method two advantages may be noted: It is not necessary to make a special puncture, thus inconvenience for the patient and physician is avoided; and the definite relation of the test to the treatment permits observations on the effect of the latter.

Obtaining the Serum.—The serum is obtained by centrifugalization of the clotted blood in the Wassermann tubes. A spontaneous retraction of the clot seldom occurs. It is, therefore, necessary to stir up the clotted blood to be sure that no fibrin adheres to the wall. There is no danger of breaking up the erythrocytes and thereby contaminating the serum, as has frequently been discussed, because the normal resistance of the red cells is sufficiently great. The destruction is much more likely to occur when hypotonic solutions, such as distilled water, come in contact with the blood. The supernatant serum is poured into a clean tube and again centrifugalized. The remaining red cells will afterward adhere to the bottom of the tube and the serum may be poured cleanly into another tube. A few cells, or even a trace of hemoglobin, which may remain in the serum, does not affect the test. Care is taken to stopper the tubes immediately after each manipulation.

Viscosimetric Examination.—If possible, the examination should be carried out in a room with a temperature of 20 C. (68 F.). Slight deviation from this temperature may be ignored, but the temperature, as shown by the thermometer between the two tubes of the viscosimeter, should be noted carefully. In order to apply the viscosimetric findings on the charts of Naegeli, the samples of serum should be measured at 20 C., or at least be corrected to this degree. If the temperature lies within 17 and 23 C., correction is not needed. The variation of viscosity with temperature is only fairly constant at the usual room temperature. Accurate determinations of human serum at various temperatures have not been published. From our experiments on the variation of viscosity with temperatures, we have adopted the following manner of correction:

For samples of serum of viscosity from 1.60 to 1.70, the correction is 0.01 for each 5 degrees Centigrade above 20 C.; for viscosity from 1.70 to 1.80, the correction is 0.01 for each 4 degrees Centigrade above 20 C.; and for viscosity from 1.80 to 1.90, the correction is 0.01 for each 3 degrees Centigrade above 20 C. For example: If at 28 C. the viscosity is 1.76, correction for 8 degrees Centigrade above 20 C. is 0.02. The viscosity of this serum at 20 C. is, therefore, 1.78.

When serum is used, cleaning the viscosimeter is not required; on the contrary, it is advantageous that the walls of the tube be wet by a fluid of about the same viscosity as that which will next be examined. The serum is, therefore, expelled slowly in order to empty the tube as nearly as possible. The next sample is drawn in, and the measurement is taken. It may be checked as often as is desired. It is possible to make 100 determinations within an hour (Table 5).

In order to obtain the second decimal of the viscosimetric reading with the instrument of Hess, which in the ordinary instruments can be obtained by estimation only, serum is drawn to the figure 2, and the

reading is divided by 2. An estimation of one-fifth between two lines can be made easily. By dividing it by 2 the reading can be made by tenths. For example, if the serum is drawn up to the figure 2, and the water column stops between the figures 3.6 and 3.7, and the meniscus is nearly midway between the two lines, but somewhat nearer 3.7, the estimation is 3.66 and the viscosity of the serum is 3.66:2 = 1.83.

TABLE 5.—TIME REQUIRED FOR THE EXAMINATION

	One Examination, Minutes	Twenty Examinations at the Same Time, Minutes
Withdrawing blood	2	From 40 to 80
Examination:		
Obtaining serum	10	20
Viscosimetry (cleaning of instrument included)	1	20
Refractometry	2	20
Calculation	2	20
Total	15	80

Refractometric Determination.—In Table 6, the few examples found in the literature of refractometric determinations on serum at various temperatures are tabulated. They form a part of the study of Strauss and Chajes.[24] We studied the influence of the temperature on the refractivity of the serum with the aid of a system for temperature regulation. In Table 7 the temperature, with the corresponding refractive angle and the refractive index, is tabulated. The figures are average values from serial readings at the corresponding temperature over half an hour. The two series correspond as well as can be

TABLE 6.—FINDINGS PUBLISHED BY STRAUSS AND CHAJES

Sample	Temperature, C.	Angle of Refraction		Index of Refraction
		Degrees	Minutes	
1	15.0	64	18.0	1.3502
	17.5	64	19.5	1.3500
	20.0	64	21.0	1.3499
	22.5	64	22.5	1.3498
	25.0	64	25.0	1.3496
2	15.0	64	19.0	1.3501
	17.5	64	21.0	1.3499
	20.0	64	24.0	1.3497
	22.5	64	26.0	1.3495
	25.0	64	27.5	1.3494

expected in the field of experiment. We conclude that the correction for each degree Centigrade is about 1 minute. If a reading of 64 degrees 45 minutes is taken at 27.5 C., 10 minutes must be subtracted to obtain the angle at 17.5 C. The angle is, therefore, 64 degrees 35 minutes. In this example, the refractive index would have to be increased from 1.34796 to 1.34878, which is 0.000082 per degree Centi-

24. Strauss, H., and Chajes, B.: Refractometrische Eiweissbestimmungen an menschlichem Blutserum und ihre klinsche Bedeutung, Ztschr. f. klin. Med. **54**:536, 1904.

grade. It seemed to be more practical to apply the reduction to the angle instead of to the index of refraction.

Computation.—The final computations are simple. Table 4 contains the angle of refraction, the index of refraction, and the Pulfrich units. The accompanying chart is used in finding the ratio of albumin and globulin. The viscosity of the serum in question is plotted on the abscissa, Pulfrich's units on the ordinate. The crossing point lies on a curve or between two curves. Each curve is indexed by a fraction, as, for instance, 60:40, which means that albumin is 60 per cent. and globulin is 40 per cent. Every other value can be found easily by interpolation.

TABLE 7.—FINDINGS OBTAINED IN OUR EXPERIMENTS

Sample	Temperature, C.	Angle of Refraction		Index of Refraction
		Degree	Minutes	
1	17.5	64	15.5	1.35048
	20.0	64	18.0	1.35025
	26.5	64	24.0	1.34969
	30.0	64	27.0	1.34944
2	13.0	64	27.0	1.34854
	18.0	64	43.0	1.34812
	20.0	64	45.0	1.34796
	26.5	64	51.2	1.34740
	40.0	64	40.5	1.34632
3	13.0	64	26.5	1.34948
	20.0	64	34.0	1.34887
	26.0	64	41.0	1.34829

TEMPERATURE CORRECTIONS

Sample	Angle of Refraction, Minutes		Difference
	At 17.5 C.	At 27.5 C.	
1	15.5	25.0	9.5
2	42.5	52.5	10.0
3	31.5	42.0	10.5
Average			10.0

SUMMARY OF RESULTS

In the majority of cases studied in which a high globulin content was present, it was found that this value decreased with the administration of each arsphenamin injection. Because of the limitations of space, it seemed unnecessary to tabulate each case, but Tables 8, 9, and 10 are given as typical examples of this decrease in globulin, under treatment.

The viscosity of the blood, based on 174 determinations in persons known to be syphilitic was usually found to lie between 1.70 and 1.90. The lowest rate observed was 1.60 and the highest 2.05. The average was 1.79. These determinations were made before, during, and after treatment with arsphenamin.

An endeavor was made to determine whether or not there was any relation between the clinical type of untreated syphilis and the viscosity. The untreated patients have been classified under three general clinical groups, and the average viscosity calculated. The viscosity in primary

and secondary syphilis was 1.86, in tertiary syphilis, 1.83, and in syphilis of the central nervous system, 1.82. The viscosity in untreated patients is perhaps a little higher than the average, but hardly enough

TABLE 8.—LATENT SYPHILIS

Case	1921	Readings: Refractivity: Viscosity at 20 C.	Refractivity: Angle at 17.5 C.: Degree	Minutes	Refractivity: Index at 17.5 C.	Interpretation with Aid of Table 4: Pulfrich's Units	Interpolation with Figure 1: Ratio of Albumin to Globulin	Globulin Percentage in Serum: Determination Made Just Before Administration of Arsphenamin. Treatments: I	II	III	IV	V	VI
78*	3/25	1.71	64	47	1.34780	53.6	42 : 58	..	58	..	..	..	..
	4/15	1.67	64	42	1.34821	54.7	56 : 44	..	..	..	44	..	..
	4/22	1.62	64	48	1.34771	53.4	60 : 40	..	..	..	..	40	..
	4/29	1.72	64	32	1.34903	56.9	58 : 42	..	..	..	..	..	42
90†	3/29	1.88	64	20	1.35000	59.5	42 : 58	..	58	..	..	..	..
	4/ 5	1.86	64	20	1.35000	59.5	45 : 55	..	..	55	..	..	..
	4/12	1.94	64	13	1.35059	61.1	41 : 59	..	..	..	59	..	..
	4/19	1.84	64	23	1.34976	58.9	46 : 54	..	..	..	..	54	..
	4/26	1.92	64	10	1.34085	61.8	47 : 53	..	..	..	..	..	53

* Wassermann reaction positive. † Wassermann reaction negative.

TABLE 9.—TERTIARY SYPHILIS

Case	1921	Readings: Refractivity: Viscosity at 20 C.	Refractivity: Angle at 17.5 C.: Degree	Minutes	Refractivity: Index at 17.5 C.	Interpretation with Aid of Table 4: Pulfrich's Units	Interpolation with Figure 1: Ratio of Albumin to Globulin	Globulin Percentage in Serum: Determination Made Just Before Administration of Arsphenamin. Treatments: I	II	III	IV	V	VI
158*	4/ 5	1.85	64	35	1.34878	56.2	29 : 71	71	..	..	..	..	..
	4/12	1.78	64	42	1.34821	54.7	33 : 67	..	67	..	..	..	..
	4/19	1.71	64	54	1.34718	52.0	29 : 71	..	..	71	..	..	..
	4/26	1.72	64	45	1.34796	54.1	42 : 58	..	..	..	58	..	..
	5/ 3	1.70	64	46	1.34788	53.8	45 : 55	..	..	..	..	55	..
181†	4/ 8	1.79	64	32	1.34903	56.9	45 : 55	55	..	..	..	..	..
	4/15	1.79	64	26	1.34952	58.2	51 : 49	..	49	..	..	..	..
	4/22	1.75	64	23	1.34976	58.9	62 : 38	..	..	38	..	..	..

* Wassermann reaction positive. † Wassermann reaction negative.

TABLE 10.—CEREBROSPINAL SYPHILIS

Case	1921	Readings: Refractivity: Viscosity at 20 C.	Refractivity: Angle at 17.5 C.: Degree	Minutes	Refractivity: Index at 17.5 C.	Interpretation with Aid of Table 4: Pulfrich's Units	Interpolation with Figure 1: Ratio of Albumin to Globulin	Globulin Percentage in Serum: Determination Made Just Before Administration of Arsphenamin. Treatments: I	II	III	IV	V	VI
82*	3/25	1.83	64	39	1.34847	55.3	27 : 73	73	..	..	..	..	..
	4/ 1	1.87	64	25	1.34960	58.5	38 : 62	..	62	..	..	..	..
	4/15	1.89	64	14	1.35051	60.8	47 : 53	..	..	..	53	..	..
	4/22	1.87	64	13	1.35059	61.1	52 : 48	..	..	..	..	48	..
	4/29	1.84	64	18	1.34017	60.0	52 : 48	..	..	..	..	..	48
92*	3/25	1.87	64	38	1.34854	55.6	21 : 79	79	..	..	..	..	..
	4/ 1	1.69	64	49	1.34763	53.1	43 : 57	..	57	..	..	..	..
	4/ 8	1.79	64	31	1.34912	57.1	46 : 54	..	..	54	..	..	..
	4/15	1.78	64	34	1.34887	56.5	46 : 54	..	..	..	54	..	..
	4/22	1.76	64	30	1.34920	57.3	48 : 52	..	..	..	..	52	..

* Cerebrospinal fluid positive.

to be of much significance. As to the relation between the clinical types and the viscosity, nothing can be deduced. Accepting as the average the viscosity of normal blood, we concluded that the blood

viscosity in itself is of little significance in cases of syphilis either as an aid to diagnosis or to differentiation of clinical types.

The refractive index was also found to be within normal limits in the cases studied. It is interesting to compare our figures with those of Winternitz.[25] He stated that a syphilitic infection is followed by an increase in the refractometric index, and that this increase goes on during the progress of the disease. We were not able to corroborate his findings (Table 11).

TABLE 11.—Comparison of Refractivity of Serum Expressed in Refractometric Units of Pulfrich

	Winternitz			Bircher		
	Highest	Lowest	Average	Highest	Lowest	Average
Normal	61.0	57.8	59.0	63.8	54.7	57.8
Primary and secondary syphilis	63.5	60.0	61.7	62.9	55.6	58.9
Tertiary syphilis	68.0	60.3	62.0	58.7	53.8	56.8

Winternitz states further that he observed a decrease of the refractivity after antispecific treatment. Our observations, which are given in Table 12, suggest, in contrast with those of Winternitz, that the refractivity of the syphilitic serum lies well within the range that might be expected under normal circumstances, and that it is not changed to any appreciable degree by treatment for syphilis. This fact is of great interest, because we shall show that the relation of viscosity and refractivity to each other is a much more delicate indicator of the pathologic process than either one alone, and that deductions from either viscosimetric or refractometric findings alone should not be made.

TABLE 12.—Refractivity of Syphilitic Serum Expressed in Refractometric Units of Pulfrich in Relation to Treatment

	Before Treatment			After Treatment		
	Highest	Lowest	Average	Highest	Lowest	Average
Primary and secondary syphilis	62.9	55.6	58.9	61.8	53.8	57.4
Tertiary syphilis	58.7	53.8	56.8	61.8	52.7	57.9

Viscosity and refractometry depend on the water content of the blood and the total protein content. The ratio between the two, however, is almost entirely independent of these factors. Naegeli states that the daily variations in the relation between viscosity and refractivity are seldom over 5 per cent., and that food intake and physical exercise have no influence on it. He also found no differences between the venous and the arterial blood.

25. Winternitz, R.: Zweiter Beiträg zur chemischen Untersuchung des Blutes rezent luetischer Menchen, Arch. f. Dermat. u. Syph. **101**:227, 1910.

There is a possibility of technical error in determining this ratio, which may reach 5 per cent., but which usually, and under constant conditions, is much smaller. The ratio as determined indicates how many parts of albumin and how many parts of globulin are present in 100 parts of serum protein. When one is stated, it is understood that the other constitutes the remainder of 100 parts. Thus, if the globulin is 60, it follows that the albumin is 40. For practical purposes, in this paper, the globulin values alone are given; from these the albumin values may be computed.

Previous investigations, as well as our own, proved the usual range of normal globulin to be between 20 and 40 per cent. Occasionally, it may reach 45 or 48 per cent.; it has almost never been more than 50 per cent. Even in the determinations of Epstein there are found only two instances of an increase in globulin over 50 per cent., namely, in cardiac and nephritic conditions. In several cases of cardiorenal disturbance, we also found a rate between 50 and 60 per cent. It is safe to conclude that a rate over 50 per cent. means a pathologic condition. This limit is not a sharp one, but may be used as a basis for discussion. The observations in our cases seem to be of significance:

1. Serial determinations of the serum globulin in a patient under specific treatment revealed a marked decrease in the globulin percentage as the treatment progressed. This decline was seen in thirty-one of a total of forty cases which were followed through a complete course of treatment. In five cases the value remained practically the same; and in four cases an increase was observed. The last four series consisted of only two or three determinations, so that an experimental error may explain the failure to decrease. Thus, in practically all the observations made over a period of five or six weeks, a decrease of globulin was observed, although occasionally some fluctuation occurred, as demonstrated in Tables 8, 9 and 10. Our observation corroborates the work of Noguchi and McDonagh.

It was further noticed that some patients, especially those in whom the initial globulin value was very high, responded markedly to treatment, while others showed little reduction of globulin.

In general, our results show that the globulin ratio is high in cases of syphilis. We tried to correlate our findings in order to explain several instances of normal globulin-albumin ratio. The patients were classified, therefore, in Groups 1, 2 and 3 according to the stage of disease and in Groups 4, 5 and 6, according to the reactions of their serums. The findings by groups are given in Table 13. The grouping on the basis of the stage or type of the disease has been proved of secondary interest only, although essential for comparative studies as well as for further investigations.

It can be stated that only exceptionally an untreated patient shows a normal globulin value. In fact, it was found in only three of seventy-one patients. In thirteen other patients, whose values were normal, the treatment had already been given, so that it was impossible to determine whether or not these patients had had an increased globulin content before treatment. It seemed inadvisable to compute the results on a percentage basis, but it can easily be seen that the conditions are very much the same in all the different groups.

If we call a globulin percentage which is higher than 50 a positive finding, it can be stated that more than 90 per cent. of our patients showed an increase of globulin before treatment and that a great number dropped to normal value after treatment.

TABLE 13.—GLOBULIN CONTENT IN THE BLOOD OF PATIENTS WITH SYPHILIS

	Before Treatment			After Treatment Was Begun		
	Patients Whose Blood Was Tested	Pateints with Globulin Above 50 per Cent.	Patients with Globulin Below 50 per Cent.	Patients Whose Blood Was Tested	Patients with Globulin Above 50 per Cent.	Patients with Globulin Below 50 per Cent.
Group 1: 19 patients—Primary, secondary, and tertiary syphilis	..	5	..	2	8	4
Group 2: 17 patients—Tertiary syphilis	7	7	..	10	10	..
Group 3: 49 patients—Cerebrospinal syphilis	17	15	2	32	23	9
Group 4: 19 patients—Positive Wassermann reaction on the blood	7	6	1	12	10	2
Group 5: 32 patients—Positive spinal fluid	13	11	2	19	13	6
Group 6: 39 patients—Negative Wassermann reaction on the blood and negative spinal fluid	10	10	..	29	23	6

From our results obtained in cases of syphilis, it might be inferred that the test is specific. This, however, is not the case. The globulin test must be considered in the same category as the determination of fever, the detection of albumin in the urine, and the calculation of leukocytes in the blood stream. In carrying out this work, we incidentally made tests on a series of patients who were under treatment with arsphenamin for cutaneous tuberculids and tuberculous glands of the neck. Practically all showed a high globulin content of the blood serum. The nonspecificity of the test makes its value more corroborative than absolute. This feature, however, is entirely in keeping with the trend of modern serology and blood chemistry as applied to syphilis. In other words, the laboratory diagnosis of syphilis consists almost entirely in recognizing the phenomena of body reaction to invaders in general and not on a unique and specific mechanism. The specificity of the mechanism of the Wassermann reaction was put in doubt when synthetic antigens were prepared from tissue lipoids. This was shortly followed by the clinical observation that yaws, certain types of tuberculosis, malignant endocarditis, and even a lipoid-loaded blood stream after a heavy meal might all

produce positive Wassermann reactions. The ordinary globulin determination in the spinal fluid may be positive in many conditions other than syphilis, and a pleocytosis in the spinal fluid is by no means pathognomonic of a syphilitic infection.

The globulin content of the blood, therefore, is probably only an index of the body's reaction against a chronic infection and is to be considered only as corroborative evidence of syphilis.

It may be said, however, that from our limited series of cases it appears that the globulin test is more constant and less subject to fluctuations than are the tests, such as the Wassermann reaction, now commonly employed. It has been the experience of every syphilographer that the serum Wassermann test and even the spinal fluid tests may vary greatly when taken over a comparatively short period of time. It is also coming to be generally recognized that negative serology does not always mean cure and hence treatment is carried on

TABLE 14.—CORRELATION OF STATUS OF DISEASE AND CONCOMITANT GLOBULIN FINDINGS

Stage of Syphilitic Infection	Globulin
Active	Increased
Short duration	Slightly increased
Long duration	Greatly increased
Wassermann reaction on blood positive	Increased
Cerebrospinal fluid positive	Increased
Change from positive Wassermann reaction on the blood or cerebrospinal fluid to negative	Accompanied by a decrease
Infection no longer demonstrable by Wassermann and cerebrospinal fluid reactions, but probably latent	Increased
After treatment	Decreased

even in the absence of positive findings. The globulin content seems to be a more dependable factor, remaining constant until treatment is started, and then declining gradually as therapy progresses. This decline seems to progress pari passu with treatment, and should therefore be studied further as a possible indicator of therapeutic response. In the section on Dermatology and Syphilology, six injections in a series constitute an average single course. In most cases the globulin content of the blood reaches a normal level at about the fifth or sixth injection. This seems to confirm the advisability of a course of at least this length. Further studies may be of value in determining the length of time which should elapse between courses.

The objective findings of our work are summarized in Table 14, in which the status of the disease and the concomitant globulin findings are correlated. While many phases yet remain to be worked out, there seems to be a measurable relation between the administration of arsphenamin and mercury, and a possible element in the defense mechanism against the disease, in the form of the globulin content of the blood.